Under certain conditions, periodate oxidizes polysaccharides. Consequently plasma of *C. intestinalis*, *A. malaca*, or *Ph. mamillata* was incubated at pH 5.4 with sodium metaperiodate, 0.02 *M* and 0.04 *M* final concentrations. The reaction mixtures were incubated in darkness for several hours at 25 °C and then dialyzed against PBS. The hemagglutinating activity of the plasma preparations was not affected by periodate treatment. The activity of the periodate concentrations was verified by incubating reaction mixtures for 1 h in which the plasma was replaced by 0.01 *M* glucose or saccharose solutions and 1% starch solution. The periodate consumption was traced by reading the absorbance at 223 nm[16].

Since molecules with definite size, shape and charge can be characterized by their solubility in a given medium, attempts were made to fractionate hemagglutinins on the basis of their solubility in ammonium sulphate solutions[17]. The experiments were carried out with plasma from *Ph. mamillata* and *A. malaca* which have shown the highest hemagglutination titres. Aliquots were incubated at 0 °C for several hours with increasing concentrations of ammonium sulphate (25, 50, 75, 100% final concentration) and then centrifuged at 18,000 × *g* for 60 min at 0 °C. The precipitate was dissolved in saline. All the samples were dyalized against PBS and then subjected to a 4-fold concentration in a Diaflo equipped with UM2 membrane (Amicon Corp., Lexington, Mass.). The active fraction precipitates completely at 75 and 100% ammonium sulphate saturation levels and the hemagglutinating activity was found in the precipitate.

In a further experiment, we attempted to see whether or not 2-mercaptoethanol, a reagent that breaks the S-S linkage in protein molecules, affects hemagglutinating activity of the plasma preparations. Aliquots of the plasma were treated for 1 h at room temperature with 2-mercaptoethanol (0.1 *M* and 0.2 *M* final concentration) in saline; the reaction was stopped by addition respectively of 0.2 *M* and 0.4 *M* iodoacetamide, and the reaction mixture were dialyzed against PBS. Both 2-mercaptoethanol concentrations destroyed the hemagglutinating activity of the plasma.

An attempt was made to digest plasma preparations with proteolytic enzyme to see if the fraction carrying the hemagglutinating activity was protein; 0.9 ml plasma preparations were dialyzed overnight against 0.05 *M* KCl-HCl buffer (pH 2) and then incubated with 0.1 ml pepsin (Sigma) solution (4 mg/ml) at 37 °C for 1 h. The reaction mixture was dialyzed against PBS and the hemagglutinating tests were performed at 37 °C or 4 °C. This treatment completely destroyed the hemagglutinating activity of plasma preparation from *Ascidia malaca* and *Phallusia mamillata*. Since the hemagglutinating activity was not influenced when enzyme-free reaction mixture were incubated, possibly hemagglutinin is digested by the enzyme.

Conclusions. Data presented in this paper show that *Ciona intestinalis* L., *Ascidia malaca* Fraust and *Phallusia mamillata* Cuv. possess in their plasma agglutinins for a number of vertebrate erythrocytes. In each Ascidian species, the agglutinins are specific for a given variety of erythrocytes. Absorption tests suggest that the reactive sites of these molecules have similar properties.

Plasma from *Phallusia mamillata* shows the highest activity and agglutinates erythrocytes from all vertebrate employed. By contrast plasma from *Ciona intestinalis* presents the lowest spectrum of hemagglutinating activity. Possibly there are small phylogenetic differences in the molecular structure of the hemagglutinins which could account for this range of specificity.

Other investigators[13] found that hemagglutinin of *Styela plicata* is very heat-stable (140 °C for 30 min), resistant to trypsin digestion and destroyed by periodate. Therefore they suggest that this molecule is a polysaccharide or a mucopolysaccharide.

Our results, obtained by physical and chemical treatments of the plasma, exclude the possibility that hemagglutinins from *Phallusia mamillata* and *Ascidia malaca* are polysaccharides. They are resistant to periodate, sensitive to mercaptoethanol, digested by pepsin, inactivated by heating at 100 °C and precipitated by ammonium sulphate. These data suggest that, at least in these two Ascidian species, the hemagglutinin may be a protein or a protein-like substance in which the molecular structure is characterized by a high molecular weight, high resistance to thermal denaturation and insensitivity to pH and cation action.

Summary. Plasma from *Ciona intestinalis*, *Phallusia mamillata* and *Ascidia malaca* possess hemagglutinin for a variety of erythrocytes. Results obtained by physical and chemical treatments suggest that hemagglutinin for *Phallusia mamillata* and *Ascidia malaca* may be a protein or a protein-like substance.

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Bio-Electrical Changes Induced by a Reaction Between Rabbit Ig and Anti-Rabbit Ig on the Surface of the Rat Cervical Ganglion

The present experiment concerns the initial phase of activation of the cell by immunological means, and is based on the following inferences drawn from immunosciences and neuro-sciences: the recognition of antigen by lymphocytes is mediated by immunoglobulin molecules incorporated into the membrane surface[1]; there is a close relationship between membrane potentials and macromolecules of the neuronal membrane; the anti-brain antibodies combine specifically with antigenic determinants of the neuronal membrane[2], and the basic processes underlying the response of the lymphocyte and the neuron to stimuli are similar[3].

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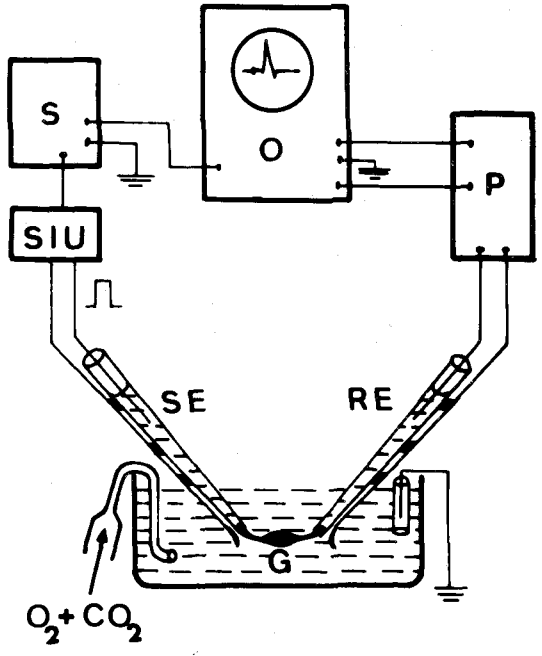

Fig. 1. Scheme of experimental assembly. The isolated rat superior cervical ganglion (G) is maintained at 37 °C in a 2 ml Perspex chamber filled with Krebs solution continuously aerated with 95% O_2 and 5% CO_2. The cervical sympathetic nerve end is aspirated into the stimulating (SE), and the carotid nerve end into the recording (RE) Ag-AgCl electrode. Square wave pulses of supramaximal strength of 6–10 V and of 0.25 sec duration, generated by a Grass S88 stimulator (S) via a stimulus isolation unit (SIU), are employed for the stimulation of the pre-ganglionic nerve fibres. Summarized ganglionic potentials are recorded on a Tectronix 502A osciloscope (O) after amplification by a low-level preamplifier (P).

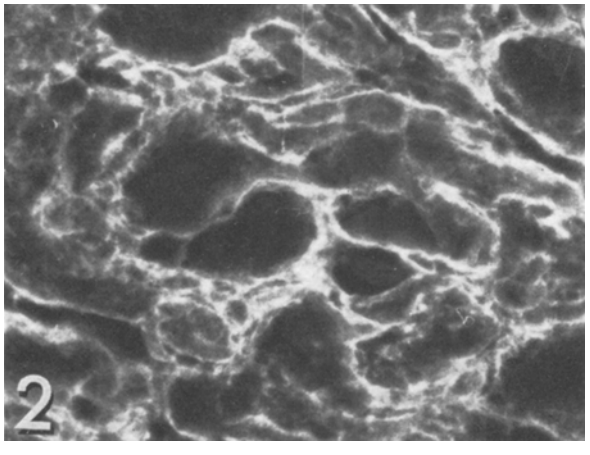

Fig. 2. Fluorescence microphotograph (×500) of a paraffin section of the rat superior cervical ganglion treated first with rabbit anti-rat synaptic membrane Ig and then with fluorescein conjugated sheep anti-rabbit Ig. Note the fluorescing neuronal membranes indicating the localization of rabbit Ig, and the absence of cytoplasmic and nuclear staining. Control sections of the rat liver and kidney treated in an identical manner were negative. No staining was observed when Ig of normal rabbit serum was used as primary reagent or when fluorescein conjugate was treated with ganglion sections that had not been pretreated with rabbit anti-synaptic membrane Ig.

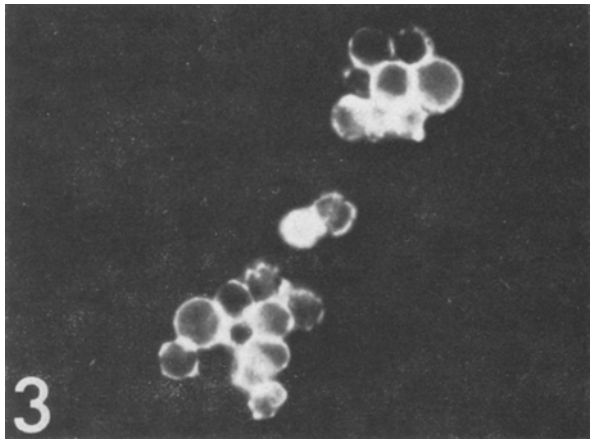

Fig. 3. Groups of brightly fluorescing rat thymocytes (×600) treated with rabbit anti-rat synaptic membrane Ig and fluorescein conjugate. The great majority of thymocytes exhibited ring-type and diffuse fluorescence.

The immuno-neurological model used in this study has 3 components: A, the membrane-associated antigens of the rat superior cervical ganglion; B, the rabbit anti-rat synaptic membrane Ig with its anti-A activity, and C, the chicken anti-rabbit Ig serum whose activity is directed against B. Functionally, antigenic determinants of the synaptic membrane of A serve a specific receptors for B, and B by virtue of its chemical nature (rabbit Ig) is a specific receptor for C. It was expected that the interaction between C and B would generate a triggering signal, send it via A to the neuronal membrane, thereby causing changes in bioelectrical discharges and activating the neuron. Thus, in this immunoneurological model the neuronal membrane serves as a biological indicator of a reaction between Ig receptors (rabbit Ig) and their functional partners (anti-rabbit Ig). The present experiment differs from studies which were primarily concerned with structural and functional dissection of the nervous system by means of anti-brain antibodies[2].

Materials and methods. The isolation of synaptic membrane[4] from the rat brain and the preparation of rabbit anti-rat brain synaptic membrane serum are described elsewhere[5]. This aniserum exhibited a titer of 1:2,000 in complement fixation reaction[6], and antibody nitrogen value of 236 ± 28 µg/10 mg of protein[7], and formed one precipitin line with corresponding antigen in agarose[8]. The chicken anti-rabbit Ig serum tested with rabbit Ig produced one precipitin band in agar and yielded 450 ± 38 µg AbN/10 mg of protein. Both antisera were absorbed with rat and/or rabbit erythrocytes, liver cell membranes and serum proteins. The Ig was separated from rabbit anti-synaptic membrane serum and dissolved in Krebs solution at 37 °C for the treatment of isolated

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Amplitudes of surface action potentials of isolated rat superior cervical ganglia treated first with rabbit anti-rat synaptic membrane Ig and then with chicken anti-rabbit Ig

No. of ganglia tested	First treatment		Second treatment		
	Reagent used	Amplitudes after washing in Krebs	Reagent used	Amplitudes after	
				1 min	5 min
7	Rabbit anti-rat synaptic membrane Ig	3.97 ± 0.30	Chicken anti-rabbit Ig	4.99 ± 0.45 ($p < 0.05$)	5.27 ± 0.57 ($p < 0.05$)
6	Rabbit anti-rat synaptic membrane Ig	4.10 ± 1.30	Normal chicken Ig	4.15 ± 1.18 ($p < 0.5$)	4.18 ± 0.1 ($p < 0.1$)
5	Normal rabbit Ig	4.52 ± 0.63	Chicken anti-rabbit Ig	5.04 ± 0.70 ($p < 0.2$)	4.88 ± 0.56 ($p < 0.6$)
5	Normal rabbit Ig	5.27 ± 1.35	Normal chicken Ig	5.45 ± 0.66 ($p < 0.3$)	5.30 ± 0.59 ($p < 0.6$)

Figures give means of amplitudes in mV $\pm$ SD. Values compared: amplitudes after the first treatment against amplitudes after the second treatment. The Student's t-test was employed.

ganglia. Paraffin sections of the rat cervical ganglion were used in fluorescent antibody assays[9]. Rat thymocytes were also exposed first to rabbit anti-synaptic membrane Ig and then to fluorescein conjugated sheep anti-rabbit IgG. The surface ganglionic potentials of the isolated rat cervical ganglion[9] were recorded as shown in Figure 1.

Results and discussion. In immunofluorescent assays, the neuronal membranes exhibited bright fluorescence whereas intracellular particulates remained unstained (Figure 2). Rat thymocytes also showed a specific fluorescence of ring-type after being exposed to anti-synaptic membrane Ig (Figure 3), a finding which supports the view that the lymphocyte and the neuron share some antigens[10,11]. A full account of this is given elsewhere[5].

In immunoneurological assays, the experimental group of 7 ganglia was exposed first to 200 µg of AbN of anti-synaptic membrane Ig in 2 ml of Krebs solution, and, after an incubation of 30 min, the ganglion was thoroughly washed with Krebs solution and the action potentials were repeatedly recorded until a stable response to stimulation was obtained. Krebs solution was then replaced by 2 ml of a Krebs solution containing 100 µg of AbN of chicken anti-rabbit Ig, and orthodromic stimuli were applied to the cervical sympathetic trunk. 3 control groups of ganglia (Table) were treated in a similar way.

Results summarized in the Table show that ganglionic cells coated with rabbit anti-synaptic membrane Ig as a rule displayed higher amplitudes of membrane potentials, ranging from 25 to 41% above their initial values, after the addition of chicken anti-rabbit Ig. This increase of ganglionic potentials occurred within 15 sec and remained at the same level for 5 min at least. Surface potentials of control ganglia did not exhibit significant changes. All action potentials demonstrated characteristics of a response to a single presynaptic volley[12].

These results suggest that the reaction between rabbit Ig (which is bound by specific forces to the neuronal membrane) and anti-rabbit Ig exerts an immediate stimulating effect on the neurons of the rat sympathetic ganglion. In the light of these findings, one may speculate about the initial phase of a specific activation of the lymphocyte by antigen. It has been shown that anti-Ig antibodies stimulate lymphocytes[13]. The energy produced by an antigen-antibody reaction[14] may be of such an amount as to cause physicochemical changes in the surface membrane and subsequent potentiation of the cell's bioelectrical activity. Thus, altered membrane potentials may be considered an integral part of the triggering signal for a specific activation of the lymphocyte. Such an activation of the lymphocyte probably differs from a nonspecific stimulation of lymphocytes by phytohaemagglutinin[15]. The immunoneurological test described can be made more sensitive if microelectrode technique is applied on simpler systems such as snail ganglion, and giant axons of the lobster, squid and *Aplysia*[2].

Résumé. Une augmentation de l'amplitude du potentiel d'action est observée dans le ganglion cervical supérieur du rat quand des Ig de lapin liées à la surface du ganglion réagissent avec des anticorps dirigés contre les Ig du lapin.

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